The concept of contexts in pain: generalization of contextual pain-related fear within a *de novo* category of unique contexts

Ann Meulders[1, 2, 3*] & Marc Patrick Bennett[4, 5]

[1] Research Group Health Psychology, KU Leuven, Leuven, Belgium

[2]Center for Excellence on Generalization Research in Health and Psychopathology, KU Leuven, Belgium

[3]Research Group Behavioral Medicine, Maastricht University, Maastricht, The Netherlands

[4]Trinity College Institute of Neuroscience, Trinity College Dublin, Dublin, Ireland

[5]Centre for Learning and Experimental Psychopathology, KU Leuven, Belgium

Key words: pain-related fear; fear conditioning; fear generalization; conceptual generalization; contextual fear; category-learning

Number of figures: 3

Number of tables: 1

Supplementary material: 3 figures and 1 manipulation check output

*Correspondence concerning this article should be addressed to Ann Meulders, PhD, Faculty of Psychology and Educational Sciences, KU Leuven, Tiensestraat 102, box 3726, 3000 Leuven, Belgium. E-mail: ann.meulders@kuleuven.be, T: +32 (0)16 32 60 38, F: +32 (0)16 32 61 44.

Disclosures

Ann Meulders is a postdoctoral researcher of the Research Foundation Flanders (FWO-Vlaanderen), Belgium, (grant ID: 12E3717N). This research was also supported by a KU Leuven Centre for Excellence grant (grant ID: PF/10/005). The authors report no conflict of interest.

Abstract

The experience of unpredictable pain fluctuations can trigger anticipatory pain-related fear. When discrete predictors for pain are lacking, fear typically accrues to the broader environmental context: a phenomenon referred to as contextual pain-related fear. We examined whether conceptual similarity between discrete contexts facilitates pain-related fear generalization; this mechanism is known as category-level fear generalization. Using a voluntary joystick movement paradigm, pain-free participants performed movements in two contexts (within-subjects design); context was manipulated by varying background color screens. In the predictable context, one movement predicted pain and another did not. In the unpredictable context, two other movements never predicted pain but pain was unpredictably delivered during the context. Participants subsequently learned to categorize novel background colors (i.e. generalization contexts) as being similar to either the unpredictable or predictable pain context. Then we tested fear generalization to these novel contexts. We measured self-reported pain-related fear, expectancy and eyeblink startle. Results indicated higher pain-related fear reports, but no elevated startle responses, for generalization contexts that were trained to be similar to the original unpredictable context rather than the predictable pain context. This highlights a potential pathway through which neutral contexts can elicit pain-related fear and motivate avoidance behavior associated with chronic pain disability.

Perspective: Self-reported pain-related fear and expectancy of painful outcome in response to a context associated with unpredictable pain generalizes to perceptually distinct contexts that are trained to be conceptually similar to the unpredictable pain context. Category-level generalization may be a pathway contributing to spreading of fear and avoidance in chronic pain.

1. Introduction

Chronic pain disability is poorly predicted by pain duration/intensity or initial tissue damage alone; psychological factors, especially pain-related fear also are critical[10, 24, 28, 30]. Pain typically signals bodily harm and/or (re)-injury initiating protective responses like autonomic arousal, hyper-vigilance, and behaviors to escape/alleviate pain. Importantly, actions and situations associated with pain may become fear-evoking over time. Such associative learning is initially adaptive; individuals can preempt pain and avoid potential harm[27]. However, persistently avoiding valued routines and experiences negatively impacts physical performance (disability and disuse syndrome), mood (depression and anxiety) and sense-of-self (diminished self-worth)[28].

Evidence suggests that learning to associate antecedent proprioceptive events or situations with painful outcomes results in acquisition of fear of movement-related pain [17]. For instance, in a voluntary joystick movement paradigm, performing one arm movement (conditioned stimulus; CS+) that predicted pain (unconditioned stimulus; pain-US) induced *cued pain-related fear*, whereas performing another arm movement that was unpaired with pain (CS-) did not. If no specific movements predict the pain, then the broader context becomes associated with pain. Indeed, contexts featuring unpredictable pain episodes gradually elicit *contextual pain-related fear* (e.g. fear of work-related activities)[16, 18].

Fear of movement-related pain generalizes to novel movements (e.g. diagonal, 45° angle joystick movement, generalization stimulus; GS) that physically resemble the CS+ movement that once preceded pain (e.g. forward, 90° angle joystick movement)[18]; a phenomenon known as stimulus generalization[11, 19]. Follow-up research showed a decrement in generalized fear when the angle of GS movements became increasingly dissimilar from that of the original CS+ movement[16]. Interestingly, pain-related fear also generalizes between conceptually similar but physically distinct cues; this is known as category-level fear generalization[7]. Bennett et al.[2] established *de novo* categories in which visual cues were arbitrarily related to certain joystick arm movements and nonsense words. Consequently, participants abstracted nonsense words and movements within the same category as being similar. Nonsense words from one category were subsequently paired with pain, whereas other nonsense words were not. Fear selectively spread to movements trained to be similar as the pain-associated words, but not to those trained to be dissimilar. Additionally, Meulders et al.[15] paired exemplars from one action category with pain (e.g. “opening” a

small, rectangular, blue box with a left joystick movement) and observed that fear generalized to conceptually similar exemplars (e.g. "opening" a large, round, yellow box with a right joystick movement), but not to novel exemplars of another action category (e.g. "closing" a small, round, green box with a left joystick movement).

Research so far focused on fear of movement-related pain and its perceptual generalization; yet the generalization of contextual pain-related fear remains under-investigated. Although the conceptual pathway may be important to understand chronic pain disability, this topic is even less studied. If pain is unexpectedly experienced in one situation (e.g. shooting back pain in a loading bay), an entire class of conceptually similar situations might trigger fear (e.g. all activities in "work-related" context)[29]. To bridge this gap, we investigated spreading of contextual pain-related fear to novel contexts that were categorically similar to the fear-eliciting (unpredictable pain) context or the control (predictable pain) context. We hypothesized that contextual pain-related fear would spread selectively to contexts related to the unpredictable pain context. Pain-related fear was assessed using self-reported measures of fear and expectancy, as well as eyeblink startle responses. Fear-potentiated startle is a commonly used psychophysiological measure in the study of perceptual generalization of pain-related fear[16-18]. However, it is unclear whether eyeblink startle is sensitive to instances of category-level generalization[15]. We therefore recorded eyeblink startle potentiation as an additional measure of pain-related fear.

2. Method and materials

2.1 Participants

Forty-two pain-free individuals (35 females; $M \pm SD$ (range) = 24.29 ± 8.45 (18-60) years) were recruited via the online experimental management system of the KU Leuven. Previous research from our laboratory informed the sample size[18]. Exclusion criteria included pregnancy, current/previous history of heart disease or other cardiovascular disease, possession of electronic implants (e.g. cardiac pacemaker), chronic or acute lung disease or other respiratory disease (e.g. asthma), neurological conditions (e.g. epilepsy) or other severe medical complaints, current/previous history of psychiatric disorders (e.g. anxiety and depression), chronic pain, wrist or hand pain that hinders the painless use of a joystick and/or mouse, color blindness, and uncorrected hearing problems. Volunteers were excluded if medically advised to avoid stressful situations. The Social and Societal Ethics Committee of the KU Leuven approved the study protocol (registration number: G-2015 01 141), and all participants provided

written informed consent. Participants were remunerated with course credit (1.5 credits) or a financial compensation (€8).

2.2 Stimuli and materials

Four proprioceptive events acted as conditioned stimuli (CSs). These included horizontal (i.e. left/right) or vertical (i.e upward/downward) joystick arm movements using a Logitech Attack 3 joystick (Logitech, Newark, CA, USA). The unconditioned stimulus was a painful electrocutaneous stimulus of duration of 2 ms (pain-US) delivered to the wrist of the dominant hand through two 8 mm Sensormedic electrodes filled with K-Y gel using a commercial stimulator (DS7A; Digitimer, Welwyn Garden City, England). The physical intensity of the pain-US was calibrated for each participant ($M \pm SD$ mA = 40.10 ± 24.70). Starting at 1 mA, a series of electrocutaneous stimuli were administered with increasing intensity and in steps of 2–3 mA. After each stimulus, participants rated the subjective intensity/painfulness using an 11-point Likert scale. Here, 0 = *"You don't feel anything at all",* 1 = *"You feel something, but it's not painful, it's merely a sensation"*, 2 = *"The sensation starts to become aversive, but it's still not painful"* and 10 =*"This is the worst pain you can imagine"*. Instructions stated that we aimed for "*a stimulus is significantly painful and demands some effort to tolerate",* and that this referred to a score of 8 on this pain intensity rating scale but that this value was not binding ($M \pm SD$ subjective intensity = 7.90 ± 0.42)[14].

The experimental context, in which CS movements were performed, was manipulated using background screen colors (defined based on the Red Green Blue (RGB) color spectrum). Black (RGB: 0.0.0) and white (RGB: 255.255.255) background screen colors were used during the acquisition of cued and contextual pain-related fear (see Figure 1A). Four other background screen colors were later used as generalization contexts. These included light grey (RGB: 68.68.68), dark grey (RGB: 191.191.191), blue (RGB: 0.80.159) and yellow (RGB: 0.80.159) screen colors (see Figure 1B).

Fear-potentiated startle (FPS) reflex provided a psychophysiological index of pain-related fear. A 50 ms burst of white noise (100 dBA) with instant rise time was used to elicit eyeblink startle (i.e. a startle probe). Startle probes were delivered binaurally through a pair of Philips SHP2500 headphones (Philips Consumer Electronics, Amsterdam, Netherlands). Responses to startle probes presented during the CS movements were used as an index of cued pain-related fear, whereas responses elicited by startle probes presented during the intertrial interval (ITI)

when only the background screen colors and counter bars were presented served as an index of contextual pain-related fear.

2.3 Experimental setting

Stimulus presentations and response recordings were programmed using Affect 4.0[23]. Stimuli were presented using a 17-inch computer screen (1024 x 768 pixels). During the experiment, participants sat in an office chair, roughly 70 cm from the computer screen, inside a sound-attenuated and dimly lit cubicle. The joystick was positioned in front of the participant, approximately 25 cm from the participant's torso. The experimenter sat in an adjacent room. Participant and experimenter could communicate through an intercom system. A closed circuit TV installation allowed for online monitoring of participants and their eyeblink startle responses.

2.4 Procedure

The experiment lasted 1.5 hours and consisted of 6 phases: (1) a preparation phase, (2) a practice phase, (3) a startle habituation phase, (4) a pain-related fear acquisition phase, (5) category learning phase (matching-to-sample task), and (6) a contextual pain-related fear generalization phase. Table 1 illustrates the experimental design.

2.4.1 Preparation phase

Upon arrival to the laboratory, participants were informed that the experiment involved the repeated presentation of electrocutaneous stimuli (pain-USs) and short loud noises (acoustic startle probes). It was also stated that involvement was completely voluntary and participants could withdraw at any stage without consequence. After informed consent was signed, electrodes for administering the pain-US were attached to wrist of the participant's dominant hand. Electrodes to record the startle responses were then attached to the forehead and beneath the left eye according to the site specification of Blumenthal et al.[3]. Finally, the calibration procedure of the pain-US intensity was initiated (see 2.2 Stimuli and materials).

2.4.2 Practice phase

Before starting the practice phase, participants received extensive written instructions about the experimental task. In each block, participants were requested to move the joystick eight times as quickly and accurately as possible when prompted by a starting signal, "+" (i.e. a red fixation cross presented in the middle of the computer screen). The position of *counter bars* on the computer screen indicated in which movement plane (horizontal *vs*. vertical) they were to move. The counter bars, each divided in four equal segments, always appeared on two sides of the computer screen (left/right or top/bottom). In a horizontal block, these bars were displayed on the left and right side of the computer screen, whereas in the vertical block, these bars appeared at the top and the bottom of the computer screen. The background color of the computer screen varied along with the horizontal/vertical blocks. For half of the participants the horizontal blocks had a white background screen and the vertical blocks a black background screen, while for the other half of the participants this order was reversed. On every trial, a green border appeared around a counter bar to indicate the direction in which participants had to move the joystick (i.e. a *direction-signal*; see Figure 1A). Successful movements always resulted in coloring one segment of that counter bar blue. That way, participants could ascertain (i) when a movement was complete and (ii) how many movements in each direction still were to be performed. In total, two blocks of eight trials were run in random order: one block in the horizontal plane and one block in the vertical plane.

The experimenter provided additional oral feedback to ensure participants mastered the CS movements. No acoustic startle probes or pain-USs were presented at this stage. At the end of the practice phase, participants completed the three Self-Assessment Manikin Scales (SAM)[4] to measure affective valence, arousal, and feelings of being in control during the respective CS movements.

2.4.3 Startle probe habituation

Initial responses to startle probes are relatively large and may confound the results. Startle probes were therefore repeatedly presented to establish a more stable pattern of responses. In a randomized order, 6 startle probes were presented in both acquisition contexts. Each trial lasted 10 s with an intertrial interval of 5 ± 2 s. A startle probe was presented randomly between second 1 and second 2, on second 3, or randomly between second 6 and second 8. In each context, 2 probes were delivered at each aforementioned interval. The pain-US and CS movements did not feature at this phase.

2.4.4 Pain-related fear acquisition

Acquisition trials were similar to the practice trials with two exceptions. First, oral feedback on CS movement performance was no longer given. Second, pain-USs and startle probes were presented. Participants completed 3 blocks in the predicable context (PRED; e.g. horizontal block with white background screen) and 3 blocks in the unpredictable context (UNPRED; e.g. vertical block with black background screen). Each block consisted of 8 trials; 4 left and 4 right movements, or 4 upward and 4 downward movements. During a block of PRED trials, the pain-US immediately followed the completion of one joystick arm movement on 3 out of the 4 trials (CS+; 75% reinforcement), but the pain-US never followed the CS- movement. The assignment of left/right and upward/downward movements to the context screens colors and stimulus type was counterbalanced across participants (for an exemplary trial overview see Figure 1A). During a block of UNPRED trials, none of the two joystick movements was followed by the pain-US (i.e. unpredictable CS1 = CSu1 and unpredictable CS2 = CSu2). Instead, the pain-US was delivered during the intertrial interval, when only the background context was visible, either before the movement (1–3 s before the start signal) or after the movement with a delay of 2–4 s. In total, 9 pain-USs were presented in the PRED context and 9 pain-USs were presented in the UNPRED context. Blocks were presented quasi-randomly with no more than two consecutive blocks of the same type.

One startle probe was presented on each trial. Four startle probes were presented during the intertrial intervals (ITI). Two probes sounded before the movement (1–3 s prior to the starting signal) and 2 probes sounded with a delay of 2–4 s after movement. To minimize any facilitation effects, startle probes and pain-USs were temporally segregated. Startle probes were delivered before the CS movement when the pain-US was presented after the CS movement, and vice versa. Four startle probes coincided with the movements. This included 2 probes during the CS+ (or CSu1) movement and 2 during the CS- (or CSu2) movement. In these cases, the startle probe was triggered 200 ms after the movement began.

After each block, participants rated how afraid they were to perform CS movements and completed a set of SAM ratings of affective valence, arousal, and perceived control.

2.4.5 Category learning

Using a category learning paradigm, participants learned to group the acquisition contexts with the novel (to-be-tested) generalization contexts (see Figure 1C). On-screen instructions stated that the task was to now learn the relationships between different contexts. Trials followed the structure of *a matching-to-sample task*[21, 22]. Each trial began with an image of a 17" Dell monitor (whose screen was colored either black or white) appearing on top of the computer screen (200 x 200 pixels). This is referred to as *the sample image*. After 1 s, the words "is similar to" appeared in the center of the screen. After 1 s, two images of 17" Dell monitor computer with different screen colors (e.g. G_PRED1 and G_UNPRED2, or G_PRED2 and G_UNPRED2) appeared side-by-side at the bottom of the screen. These are referred to as the *comparison images*. Participants selected one comparison image by moving the joystick to the left or right for the corresponding image. Corrective feedback followed each response: "correct" or "wrong" was presented on the screen for 2.5 s. In addition, a counter in the top-right corner of the screen read "your score is x". A correct (or wrong) response increased the total score by 1 (or reset the total score to zero). A 1 s ITI separated each trial. Across 4 types of training trials, (i) selecting the contexts G_PRED1 and G_PRED2 in the presence of PRED was trained and (ii) selecting the contexts G_UNPRED1 and G_UNPRED2 with UNPRED was trained (i.e. PRED → G_PRED1 and G_PRED2; UNPRED → G_UNPRED1 and G_UNPRED2). A block of training trials continued until 16 consecutively correct responses were made.

A brief test was administered to ensure that participants learned the category relations. Test trials were similar to the previous training trials with two exceptions. First, no corrective feedback was given; this ensured that symmetry relations were abstract and not based on direct reinforcement. Second, the former comparison images (i.e. G_PRED1(2) and G_UNPRED1(2)) were now sample images while the former sample images (i.e. PRED and UNPRED) were comparison images. In other words, we examined whether participants could spontaneously reverse the previously trained context-context relations; this is referred to as *symmetry*[21, 22]. Across 4 testing trial types, participants were expected to (i) select PRED in the presence of G_PRED1 and G_PRED2, and (ii) select UNPRED in the presence of G_UNPRED1 and G_UNPRED2. Four test trials were presented twice each in a block of 8 trials. Passing this test (≥75% correct) implies a symbolic inter-changeability between physically distinct contexts and, thus, that these contexts are categorically similar (see Figure 1C).

2.4.6 Contextual pain-related fear generalization

The generalization of contextual fear to categorically related contexts was tested across 6 blocks, one block in each context (i.e. two original acquisition contexts and four generalization contexts). Trials were similar to the acquisition phase with two exceptions. First, CSu1 and CSu2 movements featured in all contexts including the original predictable pain context (PRED). Second, to prevent extinction, the original unpredictable pain context (UNPRED) remained reinforced at the same rate as during acquisition, so 3 pain-USs were presented. Because no CS+ and CS- movements were performed anymore in the original predictable pain context (PRED), no pain-USs were presented in this context. No pain-US was presented in the novel generalization contexts (G_PRED1(2) and G_UNPRED1(2)).

2.5 Measures

2.5.1 Manipulation checks

Affective valence, arousal and control ratings. Three separate self-assessment manikin (SAM) scales measured affective valence, arousal and perceived control. Scale consisted of 5-points that pictured negative/positive valence, low/high arousal and low/high control, respectively[4]. Each scale was completed with respect to the question: (i) "*How did you feel when performing the upward/downward/left/right movement?*" and (ii) "*How did you feel when the background was white/black?* Ratings were made after the practice, acquisition and generalization phases.

Category learning. The numbers of correct responses during the training and testing trials were counted for each participant. Training accuracy scores and testing accuracy score were then calculated by expressing the number of correct responses as a percentage.

2.5.2 Outcome measures

Self-reported pain-US expectancy during contexts and CS movements. Participants indicated the extent to which they expected the pain-US (i) when about to perform the required CS movements and (ii) during the context present in that moment. These questions were presented when the direction-signal appeared in the acquisition and generalization phases. The questions "*To what extent do you expect the painful stimulus to occur, when performing the signaled movement?"* and *"To what extent do you expect the painful stimulus to occur during*

this background screen color, independent of the movement you are about to perform?" appeared consecutively at the top of the computer screen. Answers were given using an 11-point Likert scale that appeared on the bottom of the screen, where 0 = "*not at all*" and 10 = "*very much*".

Self-reported cued and contextual pain-related fear. Participants indicated the extent to which they feared the (i) required CS movement and (ii) context present in that moment. Questions were presented when the direction-signal appeared in the acquisition and generalization phases. The questions "*How afraid are you to perform the signaled movement"* and *"How afraid are you of the background screen color"* appeared consecutively at the top of the computer screen. Answers were given using an 11-point Likert scale, where 0 = "*not at all afraid*" and 10 = "*very afraid*".

Eyeblink startle modulation. Three re-usable Ag/AgCl SensorMedics electrodes (4mm diameter) filled with electrolyte gel were used to measure the orbicularis oculi electromyographic (EMG) activity. The skin under the left eye and the forehead was first cleaned and peeled using an exfoliating peeling cream. Electrodes were then attached, two under the left eye and one on the forehead. Unexpected auditory events, like startle probes, evoke the eyeblink startle reflex. Raw EMG signals were amplified by a Coulbourn isolated bioamplifier, with bandpass filter (LabLinc v75-04), cut-off frequency 13 Hz (low pass filter) and 500 Hz (high pass filter) (Coulbourn Instruments, White Hall, PA, USA). A Coulbourn multifunction integrator (LabLinc v76-23A) rectified and smoothed the signal with a time constant of 20 ms. The EMG signal was digitized at 1000 Hz from 200 ms before the onset of the auditory startle probe until 1000 ms after probe onset.

2.6 Response definition and data analysis overview

2.6.1 Response definition of the startle modulation

Using PSPHA[5], a modular script-based program, we calculated the peak amplitudes defined as the maximum of the response curve within 21−175 ms after the startle probe onset. All startle waveforms were visually inspected off-line, and technical abnormalities and artifacts were eliminated using the PSPHA software. The peak amplitude of each startle response was calculated by subtracting the baseline score (averaged EMG level between 1 and 20 ms after the probe onset). The raw scores were transformed to z-scores to account for inter-individual

differences in physiological reactivity. In order to optimize the visualization of the startle data and avoid negative values on the Y-axis, T-scores– a linear transformation of the z-scores– were used in the figures. Averages were calculated for responding during CS movements and ITI separately for the predictable, the unpredictable and the generalization contexts.

2.6.2 Data analysis overview

To test our main hypotheses, we carried out a series of Repeated Measures (RM) Analyses of Variance (ANOVAs) to examine the generalization of contextual pain-related fear to novel contexts that were trained to belong to the same category as the original predictable or unpredictable context. Because we had clear a priori hypotheses, we further analyzed the data using planned comparisons. Holm-Bonferroni corrections were applied to correct for multiple contrast testing per hypothesis and dependent variable. The effect size indication η_p^2 is reported for all significant omnibus ANOVA effects, and Greenhouse-Geisser corrections were applied when appropriate (uncorrected degrees of freedom and corrected *p*-values are reported together with ε). Secondary analyses were carried out to test whether pain-related fear in response to the unpredictable movements was modulated by context. Statistical analyses for all dependent measures were run with Statistica 12 software.

3. Results

3.1 Manipulation checks

Some initial conditions were necessary to address our main research questions. First, we carried out a series of repeated measures RM ANOVAs to confirm there were no pre-acquisition differences in affective valence, arousal and control between the PRED and UNPRED context colors and between the CS movements. Second, successful contextual pain-related fear acquisition is a prerequisite for the generalization of contextual pain-related fear. We therefore performed a series of RM ANOVAs, one for each dependent variable, to confirm higher fear responding to the unpredictable context relative to the predictable context. We also tested for the acquisition of cued pain-related fear using a series of RM ANOVAs. We expected higher conditioned responses to the CS+ movement than to the CS- movement, whereas no such differences were expected between the CSu1 and CSu2. Third, we checked whether successful category learning took place during the matching-to-sample task. Previous research suggests that testing trial accuracy scores of above 75% indicates the reliable formation of stimulus categories.

3.1.1 SAM ratings

Before acquisition, no differences were found between the contexts and CS movements. After acquisition, the unpredictable context became more negative, elicited more arousal, and less feelings of being in control than the predictable context. In addition, the CS+ became more negative, elicited more arousal, and less feelings of being in control than the CS- in the predictable context. However, in the unpredictable context, no such differences were observed between the CSu1 and the CSu2. For detailed results see supplementary online material.

3.1.2 Acquisition of cued and contextual pain-related fear

Our analyses confirmed that participants acquired contextual pain-related fear. That is, the unpredictable context elicited higher fear ratings, heightened pain-US expectancy ratings and elevated startle responses compared with the predictable context. Participants also successfully acquired cued pain-related fear: self-reported fear and pain-US expectancy were higher for the CS+ than for the CS-, whereas differences in startle responses were only marginally significant. No such differences occurred between the CSu1 and CSu2. For detailed results see supplementary online material.

3.1.3 Category learning (matching-to-sample task)

A mean of 25.94 (SD = 13.84) training trials were completed. Accuracy was high (M = 87.91%, SD = 7.23), suggesting that participants easily learned to relate G_UNPRED1(2) and G_PRED1(2) contexts with UNPRED and PRED, respectively. A follow-up test subsequently confirmed that participants could reverse these relations without corrective feedback (M = 94.64%, SD = 11.41). Therefore, context backgrounds were successfully grouped into two distinct categories (i.e. PRED = G_PRED1 = G_PRED2 and UNPRED = G_UNPRED1 = G_UNPRED2). For half the participants, G_PRED contexts were yellow and blue while G_UNPRED contexts were light and dark grey. This order was reversed for the remaining participants but had no impact on training accuracy ($t(47) = 0.99$, $p = .32$) or testing accuracy ($t(47) = 0.22$, $p = .83$).

3.2 Main outcomes: Contextual pain-related fear generalization

Using RM ANOVAs, we examined whether contextual pain-related fear and pain-US expectancy ratings selectively spread to novel contexts that were categorically similar to the unpredictable context, as opposed to

novel contexts that were similar to the predictable pain context. Here, context was included as a within-subjects variable with 4 levels (PRED/G_PRED/ UNPRED/G_UNPRED); for conciseness and to reduce complexity G_PRED1 + G_PRED2 were grouped into one category "G_PRED" and G_UNPRED1 + G_UNPRED2 were grouped into another category "G_UNPRED". Startle responses were analyzed using a 2 x 3 RM ANOVA with Context (PRED/G_PRED/G_UNPRED/UNPRED) and Stimulus Type (CSu1/CSu2/CTX) as within-subjects variables.

3.2.1 Generalization of self-reported contextual pain-related fear

As expected this analysis revealed a significant main effect of Context, $F(3, 144) = 22.54$, $p<.05$, $\varepsilon=.63$, $\eta_p^2=.32$. Planned comparisons showed that, during the generalization phase, participants reported more pain-related fear in the unpredictable context relative to the predictable context, $F(1, 48) = 25.80$, $p<.001$. Importantly, and as expected, novel contexts that were categorically similar to the unpredictable context (G_UNPRED) elicited heightened pain-related fear ratings than novel contexts that were similar to the predictable context (G_PRED), $F(1, 48) = 11.18$, $p<.01$. Pain-related fear reports did not differ within the class of predictable contexts (i.e. G_PRED and PRED), $F(1, 48) = 2.00$, $p=.16$. However, the original unpredictable context (UNPRED) elicited more pain-related fear than the novel context that belonged to the same category (G_UNPRED), $F(1, 48) = 33.51$, $p<.001$. These findings overall indicate that contextual pain-related fear generalized, at least partially, to contexts from within the same *de novo* category. That is, a generalization decrement was observed (see Figure 2A).

3.2.2 Generalization of self-reported pain-US expectancy during contexts

As expected this analysis revealed a significant main effect of Context, $F(3, 144) = 42.11$, $p<.001$, $\varepsilon=.62$, $\eta_p^2=.47$. Planned comparisons confirmed that, during the reinforced generalization phase, participants expected the pain-US to occur more during the unpredictable context background relative to the predictable context, $F(1, 48) = 50.41$, $p<.001$. Importantly, and as expected, novel contexts that were categorically similar to the unpredictable context (G_UNPRED) elicited heightened pain-US expectancy than novel contexts that were similar to the predictable context (G_PRED), $F(1, 48) = 20.96$, $p<.001$. Pain-US expectancy did not differ within the class of predictable contexts (i.e. G_PRED and PRED), $F<1$. The original unpredictable context (UNPRED), however, elicited higher US-expectancy ratings relative to novel context from the same category (G_UNPRED), $F(1, 48) =$

53.45, p<.001. Therefore, it appears that pain-US expectancy generalized, partially, to contexts from within the same *de novo* category. That is, a generalization decrement was observed (see Figure 2B).

3.2.3 Generalization of contextual fear-potentiated startle

As expected, this analysis revealed a significant main effect of Context, F(3, 144) = 4.90, p<.01, ε=.91, η_p^2=.09, and a significant main effect of Stimulus Type, F(2, 96) = 16.05, p<.001, ε=.77, η_p^2=.25. The Stimulus Type x Context interaction was not significant, F<1. Planned comparisons confirmed that, during the generalization phase, participants showed elevated startle responses during the contextual probes in the unpredictable context (UNPRED) as compared to the predictable context (PRED), F(1, 48) = 12.78, p<.001 (see Figure 3). In contrast with the self-report data, participants did not show higher startle responses in the novel context that belonged to the same category as the unpredictable context (G_UNPRED) than in the novel context that belonged to the same category as the predictable context (G_PRED), F<1. Although, the contextual startle responses in G_PRED were significantly lower than those in the original unpredictable context, F(1, 48) = 9.04, p<.01. ITI startle responses in both safe contexts G_PRED and PRED did not differ, F<1, but contextual startle responses in the original unpredictable context (UNPRED) were higher than those for the novel context that belonged to the same category (G_UNPRED), F(1, 48) = 9.25, p<.01.

3.3 Secondary outcomes: Modulation of cued pain-related fear in novel contexts

In order to test whether context modulates the fear and pain-US expectancy for both unpredictable CSs, we conducted 2 (Stimulus Type: CSu1/CSu2) x 4 (Context: PRED/G_PRED/ UNPRED /G_UNPRED) RM ANOVAs. To test this hypothesis in the startle responses, a 2 x 3 RM ANOVA was performed including Context (PRED/G_PRED/G_UNPRED/UNPRED) and Stimulus Type (CSu1/CSu2/CTX) as within-subjects variables.

3.3.1 Generalization of self-reported cued pain-related fear to novel contexts

This analysis yielded a main effect of Context, F(3, 144) = 16.70, p<.001, ε=.72, η_p^2=.26, but not of Stimulus Type, F(1, 48) = 1.01, p=.32. The Stimulus Type x Context interaction also failed to reach significance, F(3, 144) = 1.71, p=.17. Planned comparisons showed that, during the generalization phase, participants reported more pain-related fear to both unpredictable CSs in the original unpredictable context (UNPRED) than in the

original predictable context (PRED), $F(1, 48) = 22.03$, $p<.001$. This suggests that technically safe movements, which never before predicted the pain-US, are feared more in a threatening context than in a safe context (see Figure 2C). Interestingly, participants also reported to be more afraid when performing unpredictable CSs in the novel context that were categorically similar to the unpredictable context (G_UNPRED) than when these movements were performed in the novel context were categorically similar to the predictable context (G_PRED), $F(1, 48) = 4.27$, $p=.044$ (after Holm-Bonferroni corrections this effect was no longer statistically significant, $p > .025$). Pain-related fear for both unpredictable CSs in both safe contexts G_PRED and PRED did not differ, $F<1$, but unpredictable CSs in the original unpredictable context (UNPRED) elicited more pain-related fear than those in the novel context from within the same category (G_UNPRED), $F(1, 48) = 23.10$, $p<.001$, indicating that there was not a perfect transfer of pain-related fear within the arbitrary context category but that there was a generalization decrement.

3.3.2 Generalization of self-reported cued pain-US expectancy to novel contexts

This analysis yielded a main effect of Context, $F(3, 144) = 17.14$, $p<.001$, $\varepsilon=.78$, $\eta_p^2=.26$, but not of Stimulus Type, $F<1$. The Stimulus Type x Context interaction also failed to reach significance, $F<1$. Planned comparisons showed that, during the reinforced generalization phase, participants expected the pain-US more when performing unpredictable CSs in the original unpredictable context (UNPRED) than in the original predictable context (PRED), $F(1, 48) = 21.38$, $p<.001$. This suggests that technically safe movements triggered higher pain-US expectancies in a threatening context than in a safe context (see Figure 2D). Interestingly, participants also reported higher pain-US expectancy when performing unpredictable CSs in the novel context that were categorically similar to the unpredictable context (G_UNPRED) than when these movements were performed in the novel context that were categorically similar to the predictable context (G_PRED), $F(1, 48) = 8.53$, $p<.01$. Pain-US expectancies for both unpredictable CSs in both safe contexts G_PRED and PRED did not differ, $F(1, 48) = 1.47$, $p =.23$, but unpredictable CSs in the original unpredictable context (UNPRED) elicited higher pain-US expectancies than those in the novel context that belonged to the same category (G_UNPRED), $F(1, 48) = 20.25$, $p<.001$. This indicates that there was not a perfect transfer of acquired fear within the arbitrary context category but that there was a generalization decrement.

3.3.3 Generalization of cued fear-potentiated startle to novel contexts

As expected this analysis revealed a significant main effect of Context, $F(3, 144) = 4.90$, $p<.01$, $\varepsilon=.91$, $\eta_p^2=.09$, and a significant main effect of Stimulus Type , $F(2, 96) = 16.05$, $p<.001$, $\varepsilon=.77$, $\eta_p^2=.25$. The Stimulus Type x Context interaction was not significant, $F<1$. Planned comparisons confirmed that, during the generalization phase, startle responses were elevated for both CSs in the unpredictable context as compared to the predictable context, $F(1, 48) = 5.40$, $p<.05$. In contrast with self-report ratings, participants did not show higher startle responses for both CSs in the novel context that were categorically similar to the unpredictable context (G_UNPRED) than in the novel context that were categorically similar to the predictable context (G_PRED), $F(1, 48) = 2.36$, $p = .13$ (see Figure 3).

4. Discussion

We investigated generalization of pain-related fear to novel, conceptually similar contexts. In order to do so, we first differentially conditioned fear to one context using an unpredictable pain conditioning procedure. In a control (predictable pain) context no contextual pain-related fear was established. Results confirmed that the unpredictable context became associated with pain, prompting higher pain-related fear and pain-US expectancy ratings as well as elevated startle responses than the predictable context. Next, participants learned to categorize one pair of novel contexts as similar to the predictable context and a second pair as similar to the unpredictable context. Finally and most importantly, we tested fear generalization to these novel generalization contexts. The core hypothesis under investigation is that generalization contexts similar to the unpredictable context, but not those similar to the predictable context, would also elicit contextual pain-related fear. The findings can be summarized as follows.

First, pain-related fear selectively generalized to contexts that were similar to the original unpredictable pain context, but not to those similar to the original predictable pain context. This pattern was evident in self-reported contextual pain-related fear and pain-US expectancy. These measures were elevated in the generalization contexts similar to the unpredictable, as opposed to the predictable, context. Eyeblink startle responses did not corroborate the self-report data. The results partially uphold our main hypothesis; contextual pain-related fear can generalize to novel, conceptually similar contexts at least as measured by self-reports.

Second, generalization contexts differentially modulated fear responding to the unpredictable joystick movements –a finding that provides further *indirect* evidence for contextual pain-related fear generalization. During the generalization phase, participants performed movements that never before predicted pain (CSu1/CSu2). These movements elicited heightened pain-related fear in the unpredictable context and this spread to similar contexts. Indeed, pain-related fear and pain-US expectancy ratings for these unpredictable CSs were higher in the unpredictable contexts and its conceptually similar contexts compared to the predictable context and its conceptually similar contexts, respectively. Eyeblink startle responses to these movements were also potentiated in the unpredictable context relative to the predictable context. There was no direct difference in startling responses to movements in the different generalization contexts. However, movements in the generalization contexts that were similar to the unpredictable context still elicited elevated startle responses compared to movements in the original predictable context. These results suggest that ostensibly safe movements can elicit pain-related fear when performed in a threatening context as well as other conceptually similar contexts.

The role of perceptual generalization in pain has received increased research attention[19]. For example, movements that never predicted pain motivate fear when proprioceptively/physically similar other movements did. Generalization is not, however, perceptually limited. A key feature of human cognition is the ability to abstract conceptual knowledge about events and/or context. So in addition to perceived sensory details, semantic information about events is encoded during a painful episode and the representation of an entire category can be associated with pain. Novel exemplars from a conditioned category can resultantly motivate generalized fear responses[2, 6, 15, 26]. This is known as *category-level generalization*, and the current study is unique as it first shows that discrete contexts can participate in broader categories and elicit generalized fear. That is, this is the first demonstration of contextual pain-related fear generalization between contexts that were physically dissimilar but conceptually alike. Rather arbitrary conceptual similarities evidently afford a broad nexus of threatening and pain-relevant contexts. Indeed, conceptual similarities arguably allow more scope for generalization, as unlike perceptual similarities they are a priori arbitrary and not restricted by physical form[8].

Our findings complement two recent experimental studies. Bennett and colleagues[2] demonstrated category-level generalization of cued pain-related fear. In this study, two *de novo* categories were established in which

nonsense words were conceptually similar to specific joystick movements. Nonsense words from one category, but not the other, were then associated with pain. Results indicated that movements from the pain-relevant category selectively evoked heightened pain-US expectancy, unpleasantness and fear ratings. Meulders and colleagues[15] also demonstrated generalization of cued pain-related fear using more ecologically valid action categories. Performing joystick movements caused boxes of varying shapes, sizes and colors to "open" or "close". Ten exemplars from one category (i.e. opening) were then paired with a pain-US while ten exemplars from the other category (i.e. closing) were not. Pain-related fear evidently generalized to novel exemplars from the pain-associated category. Overall, this study adds to the growing evidence showing the role of conceptual/semantic information in the generalization of pain-related fear.

Generalization contexts that were similar to the unpredictable context did not elicit fear to the same extent, i.e. a generalization decrement was observed. It is possible that, while contexts were easily categorized into discrete categories, individual exemplars were not interchangeable or substitutable, with one another. Indeed, and in natural categories, exemplars can share an abstract similarity but can also be differentiated based on additional perceptual and conceptual information (e.g. cats and snakes are both animals but they look differently and only one is a mammal)[9]. The potential to discriminating the individual exemplars of the pain-relevant category may have potentially resulted in the partial generalization of contextual pain-related fear. Such a decrease in conditioned fear by virtue of the increasing discriminability of events/situations is characteristic of perceptual generalization; this is often depicted as a curvilinear *generalization gradient*[20]. More importantly perhaps, is that there is evidence suggesting that chronic pain patients do not show generalization gradients like of healthy controls but instead continue to (over)-generalize despite increasing physical dissimilarities. Relative to healthy controls, fibromyalgia patients display exaggerated fear to all novel movements irrespective of the similarity with the movement that originally predicted pain[14]. When unilateral chronic hand patients were asked to predict whether a fictive hand pain patients would have pain when moving his/her hand into the depicted posture (in a scenario contingency learning task), they reported higher pain-expectancy than pain-free controls for novel hand postures that grew increasingly dissimilar from hand posture that was associated with the verbal outcome "pain"[13]. As of yet, it is unclear whether chronic pain patients also show excessive generalization of contextual-pain related fear and whether such

overgeneralization can occur via the conceptual pathway. This study is an important first step in addressing these outstanding questions.

The dissociation between eyeblink startle responses and self-report ratings merits further discussion. Interestingly, Meulders and colleagues[15] noted a similar discrepancy. In their study, and unlike the self-reported pain-related fear ratings, eyeblink startle did not differentiate between exemplars from pain-associated and safe category. Two explanations were posited which can be considered with respect our findings. On the one hand, categorization is higher-order cognitive function that requires more time and effort than sensory perception alone. It is possible that startle probes were delivered too early after movement-onset, before the categorization of the functional actions was processed. This was unlikely to have occurred in the current study. Startle probes sounded 1500–3000 ms after the onset of a context or 2000–4000 ms after movements were performed. These windows allowed ample time for contexts to be categorized. Indeed, categorization specific electroencephalogram components appear as early as 150–300 ms after the initial visual stimulus onset[12, 25].

Meulders and colleagues also suggested that incidental perceptual generalization confounded eyeblink startle responses. Boxes from the pain-associated category shared some common features with boxes from the safe category. These conditioned features might have partially elicited fear in response to safe category exemplars, leading to non-differential eyeblink responses. This was also a possibility in the current study. Generalization contexts may have partially resembled both the unpredictable and predictable contexts. The grey screens, for example, may have been seen as similar to both white screens and black screens, in respect to tone and hue. Therefore, contexts that were conceptually similar to the predictable context may have elicited some fearful arousal due to a slight perceptual similarity to the unpredictable context. Previous research has indeed demonstrated that perceptual and category-level fear generalization can occur simultaneously, creating a large array of fear-relevant events[1]. This may be a limitation of the current study. Future research should attempt to exclusively model category-level generalization by minimizing physical overlap between generalization contexts and original acquisition contexts. Such procedural precautions will make it easier to determine if eyeblink startle is sensitive to category-level generalization.

In conclusion, we observed self-reported contextual pain-related fear to generalize to contexts that were conceptually similar. Assuming that psychological learning processes contribute to functional disability[27], our findings might elaborate on the experiences of chronic pain patients. That is, category-level generalization could explain why an entire nexus of previously neutral contexts can come to motivate fearful avoidance. Evidence also suggests that fear generalization is not limited by the physical similarities between these contexts. Rather arbitrary similarities, which were established during testing, were found to facilitate generalization [26]. This implies a dramatic scope of contexts that could potentially become associated with pain. An important step for future research is to examine whether chronic pain patients evince abnormal patterns of generalization between conceptually similar contexts, and whether they show resistance to extinction of such conceptually generalized pain-related fear.

References

1. Bennett M, Vervoort E, Boddez Y, Hermans D, Baeyens F. Perceptual and conceptual similarities facilitate the generalization of instructed fear. *J Behav Ther Exp Psychiatry.* 48:149-155, 2015

2. Bennett MP, Meulders A, Baeyens F, Vlaeyen JW. Words putting pain in motion: the generalization of pain-related fear within an artificial stimulus category. *Frontiers in psychology.* 6:520, 2015

3. Blumenthal TD, Cuthbert BN, Filion DL, Hackley S, Lipp OV, van Boxtel A. Committee report: Guidelines for human startle eyeblink electromyographic studies. *Psychophysiology.* 42:1-15, 2005

4. Bradley MM, Lang PJ. Measuring emotion: The self-assessment manikin and the semantic differential. *Journal of Behavior Therapy and Experimental Psychiatry.* 25:49-59, 1994

5. de Clercq A, Verschuere B, de Vlieger P, Crombez G. Psychophysiological analysis (PSPHA): a modular script-based program for analyzing psychophysiological data. *Behavior Research Methods.* 38:504-510, 2006

6. Dunsmoor JE, Martin A, LaBar KS. Role of conceptual knowledge in learning and retention of conditioned fear. *Biological psychology.* 89:300-305, 2012

7. Dunsmoor JE, Murphy GL. Categories, concepts, and conditioning: how humans generalize fear. *Trends in Cognitive Science.* 19:73-77, 2015

8. Dymond S, Schlund MW, Roche B, Whelan R, Richards J, Davies C. Inferred threat and safety: symbolic generalization of human avoidance learning. *Behav Res Ther.* 49:614-621, 2011

9. Fields L, Reeve KF, Adams BJ, Verhave T. Stimulus generalization and equivalence classes: a model for natural categories. *Journal of the Experimental Analysis of Behavior.* 55:305-312, 1991

10. Gheldof EL, Crombez G, Van den Bussche E, Vinck J, Van Nieuwenhuyse A, Moens G, Mairiaux P, Vlaeyen JW. Pain-related fear predicts disability, but not pain severity: a path analytic approach of the fear-avoidance model. *European journal of pain (London, England).* 14:870 e871-879, 2010

11. Ghirlanda S, Enquist M. A century of generalization. *Animal Behaviour.* 66:15-36, 2003

12. Kuchinke L, Fritsch N, Muller CJ. Evaluative conditioning of positive and negative valence affects P1 and N1 in verbal processing. *Brain research.* 1624:405-413, 2015

13. Meulders A, Harvie DS, Bowering JK, Caragianis S, Vlaeyen JW, Moseley GL. Contingency learning deficits and generalization in chronic unilateral hand pain patients. *J Pain.* 15:1046-1056, 2014

14. Meulders A, Jans A, Vlaeyen JW. Differences in pain-related fear acquisition and generalization: an experimental study comparing patients with fibromyalgia and healthy controls. *Pain.* 156:108-122, 2015

15. Meulders A, Vandael K, Vlaeyen JWS. Generalization of Pain-Related Fear Based on Conceptual Knowledge. *Behavior Therapy.* 48:295-310, 2017

16. Meulders A, Vandebroek N, Vervliet B, Vlaeyen JW. Generalization gradients in cued and contextual pain-related fear: an experimental study in healthy participants. *Frontiers in Human Neuroscience.* 7:345, 2013

17. Meulders A, Vansteenwegen D, Vlaeyen JW. The acquisition of fear of movement-related pain and associative learning: a novel pain-relevant human fear conditioning paradigm. *Pain.* 152:2460-2469, 2011

18. Meulders A, Vlaeyen JWS. The acquisition and generalization of cued and contextual pain-related fear: An experimental study using a voluntary movement paradigm. *Pain.* 154:272-282, 2013

19. Onat S, Buchel C. The neuronal basis of fear generalization in humans. *Nat Neurosci.* 18:1811-1818, 2015

20. Pearce JM. A model for stimulus generalization in pavlovian conditioning. *Psychological Review.* 94:61-73, 1987

21. Sidman M. Reading and auditory-visual equivalences. *Journal of Speech, Language, and Hearing Research.* 14:5-13, 1971
22. Sidman M: Equivalence relations: Some basic considerations. In: Understanding verbal relations (Hayes, S.C., G., H.L., Eds.), Context Press, Reno, NV, 1992, pp. 15-27.
23. Spruyt A, Clarysse J, Vansteenwegen D, Baeyens F, Hermans D. Affect 4.0: a free software package for implementing psychological and psychophysiological experiments. *Exp Psychol.* 57:36-45, 2010
24. Swinkels-Meewisse IE, Roelofs J, Schouten EG, Verbeek AL, Oostendorp RA, Vlaeyen JW. Fear of movement/(re)injury predicting chronic disabling low back pain: a prospective inception cohort study. *Spine.* 31:658-664, 2006
25. Thorpe S, Fize D, Marlot C. Speed of processing in the human visual system. *Nature.* 381, 1996
26. Vervoort E, Vervliet B, Bennett M, Baeyens F. Generalization of human fear acquisition and extinction within a novel arbitrary stimulus category. *PLoS One.* 9:e96569, 2014
27. Vlaeyen JW. Learning to predict and control harmful events: chronic pain and conditioning. *Pain.* 156 Suppl 1:S86-93, 2015
28. Vlaeyen JW, Linton SJ. Fear-avoidance model of chronic musculoskeletal pain: 12 years on. *Pain.* 153:1144-1147, 2012
29. Waddel G, Newton M, Henderson I, Somerville D, Main CJ. A fear-avoidance belief questionnaire (FABQ) and the role of fear-avoidance belief in chroni low back pain and disability. *Pain.* 52:157-168, 1993
30. Wideman TH, Sullivan MJ. Differential predictors of the long-term levels of pain intensity, work disability, healthcare use, and medication use in a sample of workers' compensation claimants. *Pain.* 152:376-383, 2010

Acknowledgments

The authors would like to thank Ellen Peeters for her assistance in the data collection.

Figure captions

Figure 1. A schematic overview of the experimental stages. (**A**) Fear acquisition: In the predictable context (PRED), performing one arm movement (CS+), but not another (CS-), triggered a pain-US (i.e. an electrocutaneous stimulus to the wrist). This provided a model of cued-pain related fear. In the unpredictable context (UNPRED), CS movements did not correlate with the pain-US; instead, the pain-US occurred during the intertrial interval (ITI). This provided a model of contextual pain-related fear. (i) A green border appeared around a counter bar to indicate the required CS movement direction. (ii) Participants rated their expectancy of the pain-US with respect to the CS movement and context. (iii - iv) CS movements preceded the pain-US in the predictable context, but not the unpredictable context. (v) A segment of the counter bar turned blue to indicate the completion of a CS movement. (**B**) Generalization contexts either related to the predictable context (i.e. PRED = G_PRED1 and G_PRED2) or the unpredictable context (UNPRED = G_UNPRED1 and G_UNPRED2) were tested. (**C**) Category learning: Two *de novo* categories of contexts were established using a brief '*matching-to-sample*' paradigm. During an initial set of training trials, relating G_PRED (or G_UNPRED) contexts as similar to the original PRED (or UNPRED) context was reinforced using corrective feedback (e.g. "correct" or "wrong"). A set of test trials then probed for accurate categorization. Here, we examined whether participants would reverse the previously trained relations and relate PRED (or UNPRED) contexts as similar to the G_PRED (or G_UNPRED) contexts in the absence of corrective feedback.

Figure 2. Mean self-reported pain-related fear (±SE) during the generalization phase in response to (**A**) the original acquisition contexts (PRED and UNPRED) as well as both classes of generalization contexts (G_UNPRED and G_PRED), (**C**) the CSu1 and CSu2 in the predictable (PRED) context, the unpredictable (UNPRED) context, as well as in both classes of generalization contexts (G_UNPRED and G_PRED) separately. Mean pain-US expectancy (±SE) during the generalization phase in response to (**B**) the original acquisition contexts (PRED and UNPRED) as well as both classes of generalization contexts (G_UNPRED and G_PRED), (**D**) the CSu1 and CSu2 in the predictable (PRED) context, the unpredictable (UNPRED) context, as well as in both classes of generalization contexts (G_UNPRED and G_PRED) separately.

Figure 3. Mean eyeblink startle amplitudes (±SE) during the generalization phase for the CSu1, CSu2, and the ITI (CTX) in the predictable (PRED) context, the unpredictable (UNPRED) context, as well as in both classes of generalization contexts (G_UNPRED and G_PRED) separately. Note – that for graphic purposes T-scores were used.

Supplementary Figure S.1. Mean ratings of affective valence (**A**), arousal (**C**) and feeling in control (E) after the practice, acquisition (acq) and generalization phase (gen) for the predictable and unpredictable contexts as well as mean ratings of affective valence (**B**), arousal (**D**) and feeling in control (**F**) after the practice, acquisition (acq) and generalization phase (gen) for the CSs in the predictable and unpredictable contexts. For affective valence: lower values refer to less negative and higher values refer to more negative; for arousal: lower values indicate higher arousal and higher values indicate lower arousal; for feeling in control: lower values indicate less control and higher values indicate more control.

Supplementary Figure S.2. Mean self-reported pain-related fear (±SE) during the acquisition phase (acq1-3) in response to (**A**) PRED and UNPRED, (**C**) the CS+ and CS- as well as the CSu1 and the CSu2 for the predictable (PRED) and unpredictable (UNPRED) contexts separately. Mean pain-US expectancy (±SE) during the acquisition phase (acq1-3) in response to (**B**) PRED and UNPRED, (**D**) the CS+ and CS- as well as the CSu1 and the CSu2 for the predictable (PRED) and unpredictable (UNPRED) contexts separately.

Supplementary Figure S.3. Mean eyeblink startle amplitudes (±SE) during the acquisition phase for the CS+ (CSu1), CS- (CSu2) and the ITI (CTX) for both predictable (PRED) and unpredictable (UNPRED) context separately. Note – that for graphic purposes T-scores were used.

Figure 1.

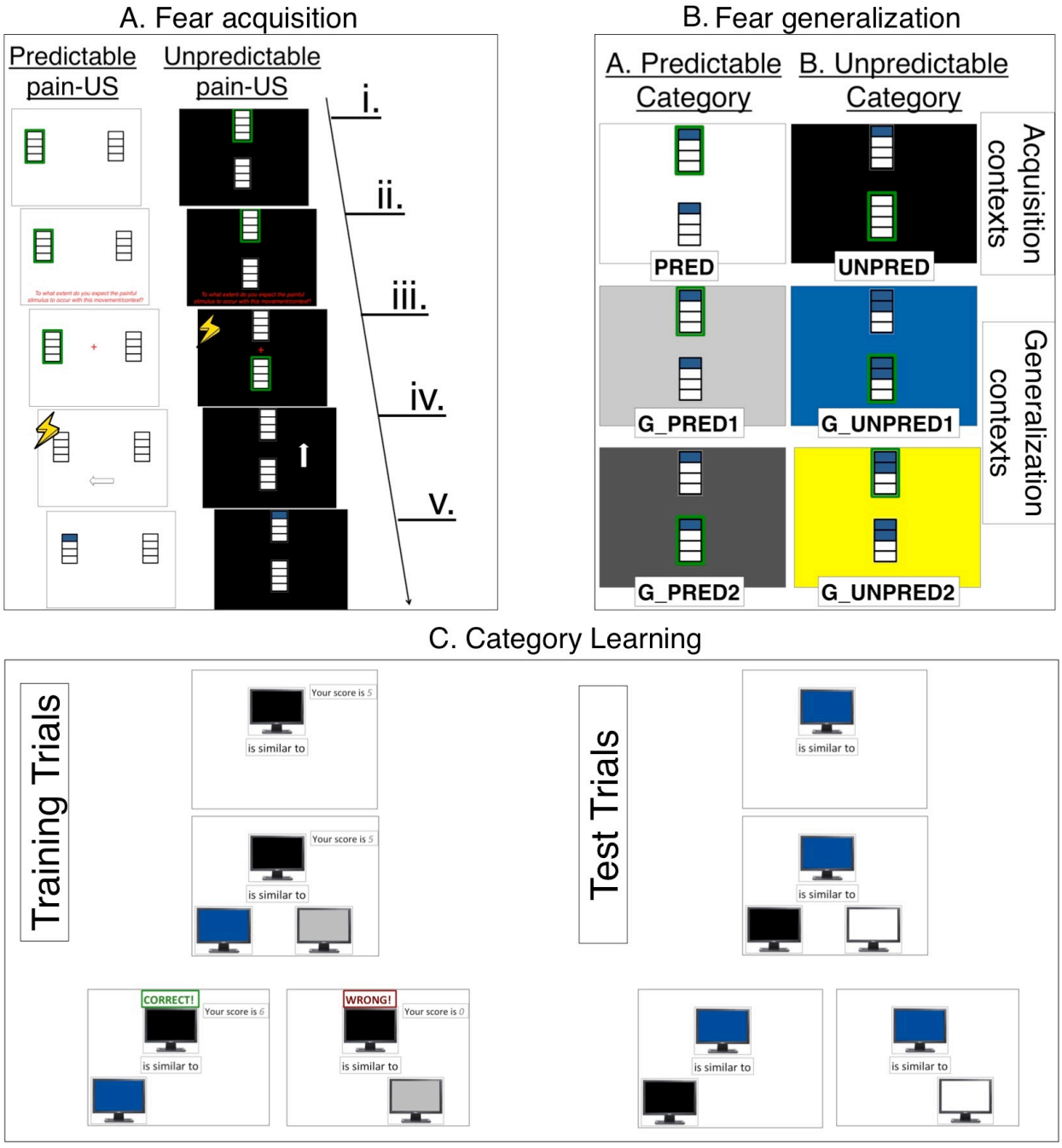
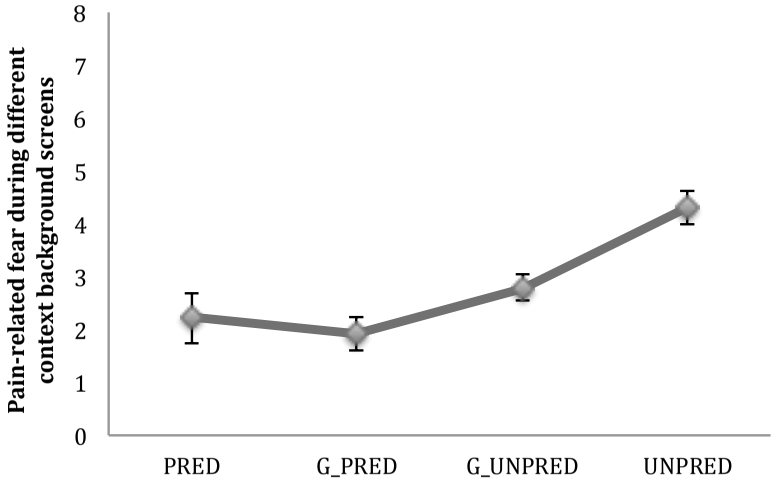
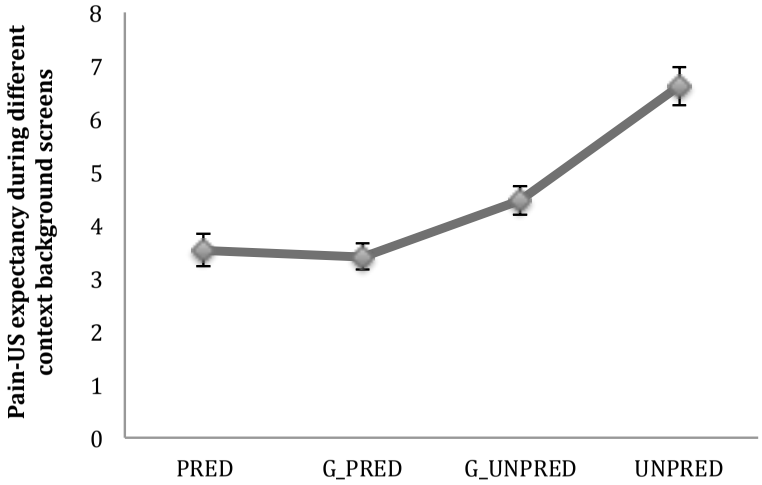

A. Fear acquisition
Predictable pain-US
Unpredictable pain-US
i.
ii.
iii.
iv.
v.
B. Fear generalization
A. Predictable Category
B. Unpredictable Category
Acquisition contexts
Generalization contexts
PRED
UNPRED
G_PRED1
G_UNPRED1
G_PRED2
G_UNPRED2
C. Category Learning
Training Trials
Test Trials
Your score is 5
is similar to
CORRECT!
Your score is 6
WRONG!
Your score is 0

Figure 2

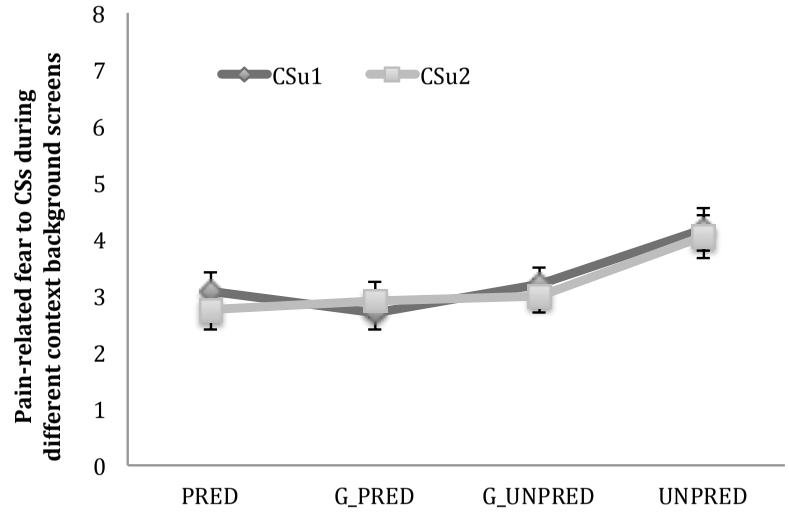
A. Generalization of contextual pain-related fear



B. Generalization of contextual pain-US expectancy



C. Generalization of cued pain-related fear



D. Generalization of cued pain-US expectancy

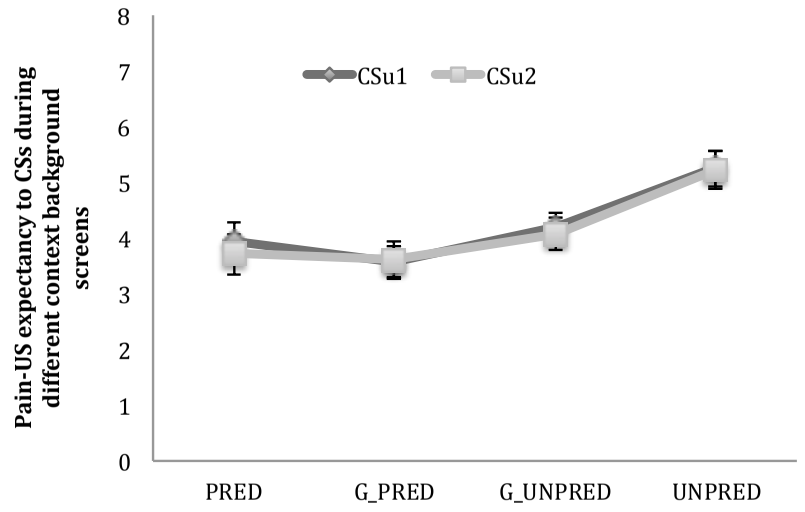


Figure 3

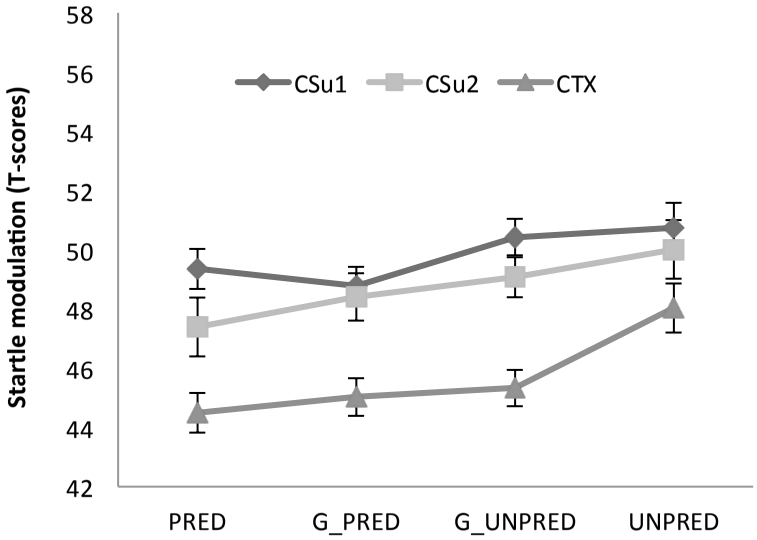
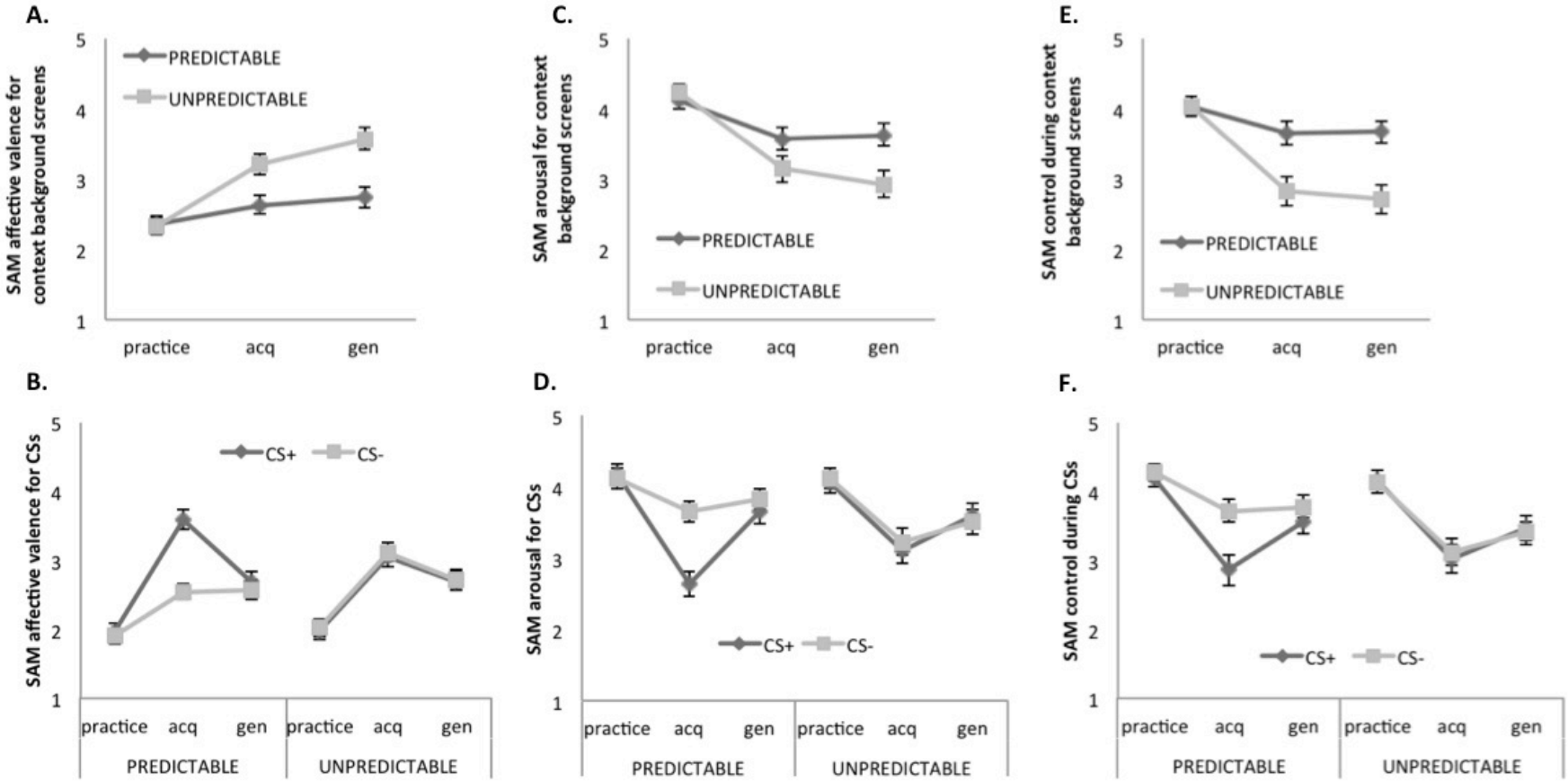

58
56
54
52
50
48
46
44
42
Startle modulation (T-scores)
CSu1
CSu2
CTX
PRED
G_PRED
G_UNPRED
UNPRED

Table 1. *Study design summary*

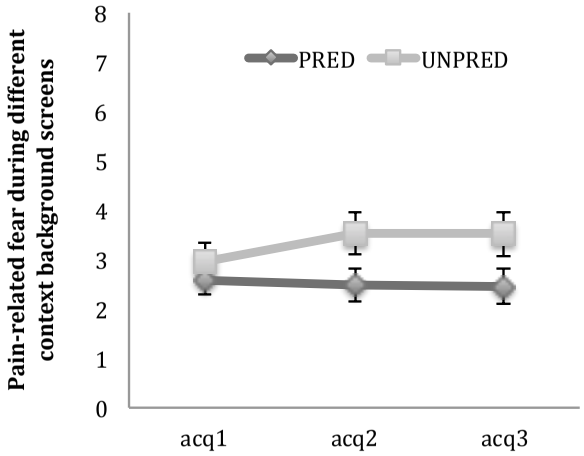
Practice phase (2 x 8 trials)	**Startle habituation phase** (12 trials)	**Pain-related fear acquisition phase** (6 x 8 trials)	**Category learning phase Matching-to-Sample** Mean (*SD)* trials = 26(14)	**Contextual pain-related generalization phase** (6 x 8 trials)
				$\begin{bmatrix} 4\,x & CSu1 \\ 4\,x & CSu2 \end{bmatrix} PRED$
				$\begin{bmatrix} 4\,x & CSu1 \\ 4\,x & CSu2 \end{bmatrix} G\_PRED1$
$\begin{bmatrix} 4\,x & CS+ \\ 4\,x & CS- \end{bmatrix} PRED$	[6 probes] $PRED$	$3\,x \begin{bmatrix} 4\,x & CS+ \\ 4x & CS- \end{bmatrix} PRED$		$\begin{bmatrix} 4\,x & CSu1 \\ 4\,x & CSu2 \end{bmatrix} G\_PRED2$
				$\begin{bmatrix} 4\,x & CSu1 \\ 4\,x & CSu2 \end{bmatrix} G\_UNPRED1$
$\begin{bmatrix} 4\,x & CSu1 \\ 4\,x & CSu2 \end{bmatrix} UNPRED$	[6 probes] $UNPRED$	$3\,x \begin{bmatrix} 4\,x & CSu1 \\ 4\,x & CSu2 \end{bmatrix} UNPRED°$		$\begin{bmatrix} 4\,x & CSu1 \\ 4\,x & CSu2 \end{bmatrix} G\_UNPRED2$
				$\begin{bmatrix} 4\,x & CSu1 \\ 4\,x & CSu2 \end{bmatrix} UNPRED°$

Note – CS = conditioned stimulus; pain-US = painful electrocutaneous stimulus (2 ms duration); CS+ and CS-, respectively, refer to the movement direction that is followed by the pain-US (75% reinforcement) and the movement that is never followed by the pain-US in the predictable context (PRED). During the practice phase, the CS+ was not reinforced. In the unpredictable context (UNPRED), the same number of pain-USs is presented during the intertrial interval (ITI). A "°" indicates reinforcement in UNPRED. When the labels PRED and UNPRED (and so on) are placed after brackets, all trials within the brackets are performed with the corresponding context. G_PRED1 and G_PRED2 are both generalization contexts that are made similar to PRED and G_UNPRED1 and G_UNPRED2 are both generalization contexts that are made similar to UNPRED.

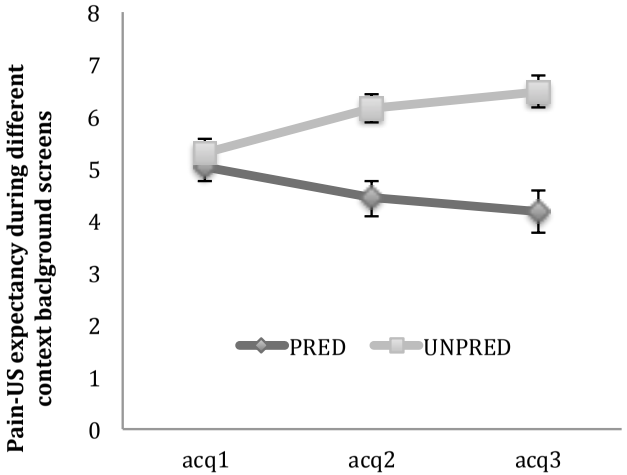

A.
SAM affective valence for context background screens
PREDICTABLE
UNPREDICTABLE
practice
acq
gen
C.
SAM arousal for context background screens
E.
SAM control during context background screens
B.
SAM affective valence for CSs
CS+
CS-
PREDICTABLE
UNPREDICTABLE
D.
SAM arousal for CSs
F.
SAM control during CSs

Supplementary Figure S.2

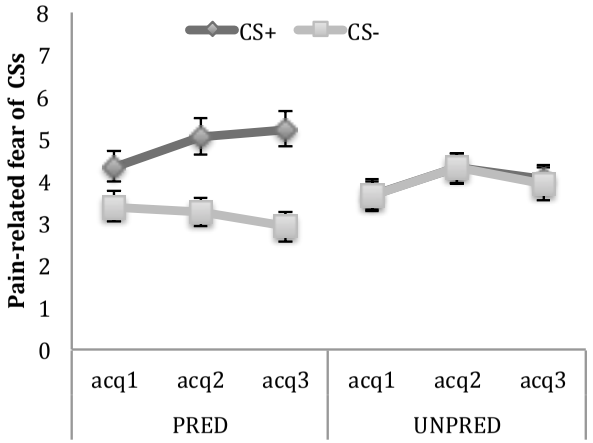
A. Acquisition of contextual pain-related fear



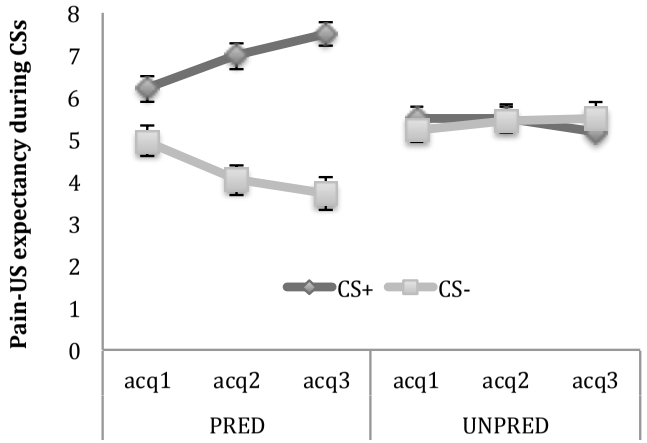
B. Acquisition of contextual pain-US expectancy



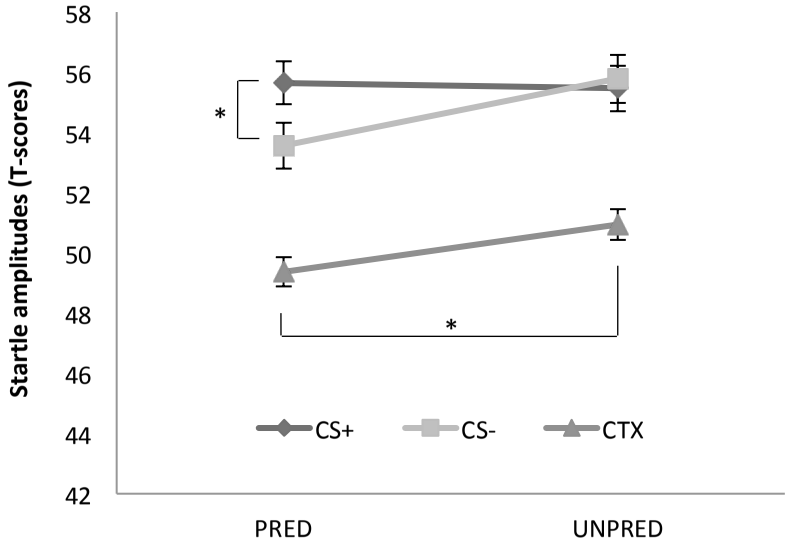
C. Acquisition of cued pain-related fear



D. Acquisition of cued pain-US expectancy



Supplementary Figure S.3



Supplementary material – manipulation checks

SAM ratings

For the three SAM ratings assessing the affective valence, arousal and perceived control for each context, we performed 2 (Context: PRED/UNPRED) x 3 (Phase: practice/acq/gen) RM ANOVAs. Next, for the three SAM ratings pertaining the CS movements similar 2 (Stimulus Type: CS+/CS-) x 2 (Context: PRED/UNPRED) x 3 (Phase: practice/acq/gen) RM ANOVAs were run.

Affective valence of the CSs and contexts.

The analysis of affective valence ratings for the contexts (see supplementary Figure S.1A) revealed significant main effects for Context, $F(1, 48) = 11.69$, $p<.01$, $\eta_p^2=.20$, and Phase, $F(2, 96) = 27.37$, $p<.001$, $\varepsilon=.90$, $\eta_p^2=.36$. Furthermore, there was a significant Context x Phase interaction, $F(2, 96) = 9.20$, $p<.001$, $\varepsilon=.88$, $\eta_p^2=.16$, suggesting that context ratings changed across the experimental phases. Planned comparisons confirmed that ratings for the predictable and unpredictable context did not differ at the end of the practice phase, $F<1$, but differed at the end of the acquisition phase. As expected, participants felt less negative about the predictable context than in the unpredictable context, $F(1, 48) = 7.25$, $p<.01$. This difference was still significant at the end of the generalization phase, $F(1, 48) = 18.57$, $p<.001$.

The analysis of affective valence ratings for the CS movements (see supplementary Figure S.1B) revealed significant main effects for Stimulus Type, $F(1, 48) = 13.51$, $p<.001$, $\eta_p^2=.22$, and Phase, $F(2, 96) = 55.09$, $p<.001$, $\varepsilon=.98$, $\eta_p^2= .53$. Also, there was a significant Stimulus Type x Phase interaction, $F(2, 96) =11.55$, $p<.001$, $\varepsilon=.86$, $\eta_p^2=.19$, suggesting that the differences between the CS+ and the CS- changed over the experimental phases. There was also a significant Context x Stimulus Type interaction, $F(2, 96) = 16.67$, $p<.001$, $\eta_p^2=.26$, suggesting differences between the CSs depending on context. These two-way interactions were accommodated by a significant three-way interaction, $F(2, 96) =14.22$, $p<.001$, $\varepsilon=.90$, $\eta_p^2=.23$. Planned comparisons confirmed no significant difference between CS+, CS-, CSu1 and CSu2 at the end of the practice phase, $F<1$. After acquisition, however, participants felt less negative about performing the CS- movement than the CS+ movement in the

predictable context, $F(1, 48) = 33.56$, $p<.001$. In contrast, participants in the unpredictable context, reported no differences in affective valence between both CSs after acquisition and generalization, both Fs<1.

Arousal elicited by the CSs and contexts.

The analysis of the arousal elicited by the contexts (see supplementary Figure S.1C) showed significant main effects for Context, $F(1, 48) = 9.04$, $p<.01$, $\eta_p^2=.16$, and Phase, $F(2, 96) = 20.15$, $p<.001$, $\varepsilon=.76$, $\eta_p^2=.30$. Furthermore, there was a significant Context x Phase interaction, $F(2, 96) = 11.06$, $p<.001$, $\varepsilon=.85$, $\eta_p^2=.19$, suggesting that context ratings changed across the experimental phases. Planned comparisons confirmed that ratings for the predictable and unpredictable context did not differ at the end of the practice phase, $F(1, 48) = 3.13$, $p=.08$. However, and as expected, participants experienced higher arousal in the unpredictable context than in the predictable context after the acquisition phase, $F(1, 48) = 6.97$, $p<.05$. This difference was still significant after the generalization phase, $F(1, 48) = 12.77$, $p<.001$.

The analysis of arousal ratings for the CS movements (see supplementary Figure S.1D) revealed significant main effects for Stimulus Type, $F(1, 48) = 17.94$, $p<.001$, $\eta_p^2=.27$, and Phase, $F(2, 96) = 19.37$, $p<.001$, $\varepsilon=.74$, $\eta_p^2=.29$. Furthermore, there was a significant Stimulus Type x Phase interaction, $F(2, 96) =28.05$, $p<.001$, $\varepsilon=.92$, $\eta_p^2=.37$, suggesting that the differences between the CS+ and the CS- changed over the experimental phases. There was also a significant Context x Stimulus Type interaction, $F(2, 96) = 12.56$, $p<.001$, $\eta_p^2=.21$, suggesting differences between the CSs depending on context. There was also a significant Stimulus Type x Phase x Context, $F(2, 96) =15.36$, $p<.001$, $\varepsilon=.78$, $\eta_p^2=.24$. Planned comparisons revealed no differences between the CS+, the CS-, the CSu1 and the CSu2 after the practice phase, F<1. After acquisition, however, participants experienced more arousal when performing the CS+ movement than the CS- movement in the predictable context, $F(1, 48) = 52.13$, $p<.001$. In contrast, participants in the unpredictable context, reported no differences in arousal between both CSs after acquisition and generalization, Fs<1.

Feelings of being in control during CSs and contexts.

The analysis on feelings of being in control during the contexts (see supplementary Figure S.1E) showed significant main effects for Context, $F(1, 48) = 27.29$, $p<.001$, $\eta_p^2=.36$, and Phase, $F(2, 96) = 18.30$, $p<.001$, $\varepsilon=.66$,

η_p^2=.36. Also, there was a significant Context x Phase interaction, F(2, 96) = 16.03, p<.001, ε=.84, η_p^2=.25, suggesting that context ratings changed across the experimental phases. Planned comparisons further confirmed that ratings for the predictable and unpredictable context did not differ at the end of the practice phase, F<1. After the acquisition phase, and as expected, participants experienced more feelings of control in the predictable context than in the unpredictable context, F(1, 48) = 19.44, p<.001. This difference was still significant after the generalization phase, F(1, 48) = 32.83, p<.001.

The analysis of control ratings for the CS movements (see supplementary Figure S.1F) revealed significant main effects for Stimulus Type, F(1, 48) = 10.87, p<.01, η_p^2=.18, and Phase, F(2, 96) = 21.74, p<.001, ε=.79, η_p^2= .31. There was also a significant Stimulus Type x Phase interaction, F(2, 96) =11.94, p<.001, ε=.85, η_p^2=.20, suggesting that the differences between the CS+ and the CS- changed over the experimental phases. There was also a significant Context x Stimulus Type interaction, F(2, 96) = 11.02, p<.01, η_p^2=.19, suggesting differences between the CSs depending on context. In addition, there was a significant Stimulus Type x Phase x Context interaction, F(2, 96) =4.10, p<.05, ε=.80, η_p^2=.08. Planned comparisons further confirmed that control ratings for CS+, CS-, CSu1 and CSu2 did not significantly differ at the end of the practice phase, F<1. After acquisition, however, participants felt more in control when performing the CS- movement than the CS+ movement in the predictable context, F(1, 48) = 18.00, p<.001. In contrast, participants in the unpredictable context, reported no differences in control between both CSs after acquisition and generalization, both Fs<1.

Acquisition of cued and contextual pain-related fear

Self-reported contextual pain-related fear.

We ran a 2 (Context: PRED/UNPRED) x 3 (Block: acq1-3) RM ANOVA on the pain-related fear reports (supplementary Figure S.2A). This analysis revealed a main effect of Context, F(1, 48) = 7.10, p<.05, η_p^2=.13. There was however no significant main effect of Block, F<1, and no significant interaction effect between Block x Context, F(2, 96) = 2.43, p =.09. Planned comparisons indicated no significant differences between the unpredictable and predictable contexts at the beginning of the acquisition phase (acq1), F<1. However, and most

importantly, participants reported more pain-related fear during the presentation of the unpredictable context relative to the predictable context by the end of acquisition (acq3), $F(1, 48) = 6.74$, $p<.05$.

Self-reported pain-US expectancy during contexts.

We ran a 2 (Context: PRED/UNPRED) x 3 (Block: acq1-3) RM ANOVA on the pain-US expectancy reports (see supplementary Figure S.2B). This analysis revealed a main effect of Context, $F(1, 48) = 16.77$, $p<.001$, $\eta_p^2=.26$, but no significant main effect of Block, $F<1$. The Block x Context interaction was also significant, $F(2, 96) = 15.79$, $p<.001$, $\varepsilon=.80$, $\eta_p^2=.25$, suggesting that differential pain-US expectancy for both contexts emerged across acquisition blocks. Planned comparisons indeed confirmed that, at beginning of the acquisition phase (acq1), expectancy ratings did not differ for the predictable and the unpredictable context, $F(1, 48) = 1.11$, $p=.30$. However, at the end of the acquisition phase (acq3), participants reported heightened expectancy of the pain-US during the unpredictable context as compared to the predictable context, $F(1, 48) = 21.38$, $p<.001$.

Fear-potentiated startle during contexts.

We ran a comprehensive analysis including both responses to the CS (movement) probes and the CTX (contextual, i.e. during ITI) probes on the fear-potentiated startle (see supplementary Figure S.3). That is, we conducted a 2 (Context: PRED/UNPRED) x 3 (Block: acq1-3) x 3 (Stimulus Type: CS+/CS-/CTX) RM ANOVA. This analysis revealed main effects for Context, $F(1, 48) = 4.81$, $p<.05$, $\eta_p^2=.09$, and Block, $F(2, 96) = 23.24$, $p<.001$, $\varepsilon=.98$, $\eta_p^2=.33$. Planned comparisons confirmed that, during acquisition, contextual startle responses were higher in the unpredictable context than in the predictable context, $F(1, 48) = 9.27$, $p<.01$. Importantly, this indicates the acquisition of contextual fear.

Self-reported cued pain-related fear.

We ran a 2 (Stimulus Type: CS+/CS-) x 2 (Context: PRED/UNPRED) x 3 (Block: acq1-3) RM ANOVA on the pain-related fear reports (see supplementary Figure S.2C). This analysis yielded a significant main effect of Stimulus Type, $F(1, 48) = 37.13$, $p<.001$, $\eta_p^2=.44$, and a marginally significant effect of Block, $F(2, 96) = 3.02$, $p=.05$, $\varepsilon=.82$, $\eta_p^2=.06$. The effect of Context was not significant, $F<1$. There was however a significant Stimulus

Type x Context interaction, $F(1, 48) = 20.96$, $p<.001$, $\eta_p^2=.30$, and a significant Stimulus Type x Block interaction, $F(2, 96) = 5.01$, $p<.01$, $\varepsilon=.96$, $\eta_p^2=.09$, which were further accommodated by a significant three-way interaction, $F(2, 96) = 3.22$, $p<.05$, $\varepsilon=.90$, $\eta_p^2=.06$. Planned comparisons confirmed that at the end of the acquisition, participants in the predictable context reported more fear when performing the CS+ movement than the CS- movement, $F(1, 48) = 36.48$, $p<.001$, whereas no such differences were found for both CSs in the unpredictable context, $F<1$.

Self-reported cued pain-US expectancy.

We ran a 2 (Stimulus Type: CS+/CS-) x 2 (Context: PRED/UNPRED) x 3 (Block: acq1-3) RM ANOVA on the expectancy (see supplementary S.2D) reports. This analysis showed a significant main effect of Stimulus Type, $F(1, 48) = 54.20$, $p<.001$, $\eta_p^2=.53$, but no significant main effects of Context or Block, both Fs<1. There was however a significant Stimulus Type x Context interaction, $F(1, 48) =35.24$, $p<.001$, $\eta_p^2=.42$, and a significant Stimulus Type x Block interaction, $F(2, 96) = 5.37$, $p<.01$, $\varepsilon=.95$, $\eta_p^2=.10$, which were also accompanied by a significant three-way interaction, $F(2, 96) = 14.34$, $p<.001$, $\varepsilon=.96$, $\eta_p^2=.23$. Planned comparisons revealed that, at the beginning of the acquisition phase (acq1), pain-US expectancy ratings did not differ between the CS+ and CS- movements. However, and at the end of acquisition (acq3), participants expected the pain-US to occur more when performing the CS+ movement relative to the CS- movement (in the predictable context), $F(1, 48) = 51.86$, $p<.001$. As expected, no such differences were found for the CSs in the unpredictable context, $F(1, 48) = 1.67$, $p=.20$.

Cued fear-potentiated startle.

The former 2 (Context: PRED/UNPRED) x 3 (Block: acq1-3) x 3 (Stimulus Type: CS+/CS-/CTX) RM ANOVA was used again to interpret cued fear-potentiated startle (see supplementary Figure S.3). Interestingly, there was a significant main effect of Stimulus Type, $F(2, 96) = 23.45$, $p<.001$, $\varepsilon=.86$, $\eta_p^2=.33$. The Stimulus Type x Context interaction was also significant, $F(2, 96) = 3.48$, $p<.01$, $\varepsilon=.99$, $\eta_p^2=.07$, suggesting that startle responses elicited by the contexts and CSs differentially progressed in both contexts. This interaction was modulated by Block, $F(2, 96) = 3.21$, $p<.05$, $\varepsilon=.88$, $\eta_p^2=.06$. Planned comparisons confirmed that, during the acquisition phase, startle responses to CS+ were higher than those for CS- (in the predictable context), $F(1, 48) = 5.19$, $p=.027$ (after

Holm-Bonferroni corrections this effect was no longer statistically significant, $p > .025$). Importantly, this finding is indicative of the acquisition of cued pain-related fear (see Figure S.3). As expected, no such difference was observed for CSs in the unpredictable context, $F<1$.